# Control of the Snake Mite, Ophionyssus natricis (Gervais), in Captive Reptile Collections<sup>1</sup>

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(Text-figures 1-3)

#### INTRODUCTION

LTHOUGH the snake mite, Ophionyssus natricis (Gervais, 1844), has been known from captive reptiles since 1823 (Metaxa, 1823), it has only recently been found on wild reptiles (Yunker, 1956). Members of the genus Coluber and its close relatives appear to be its favorite hosts in zoological gardens throughout the world and in its natural habitat in Egypt, but it will readily attack most species of snakes and many species of lizards and will occasionally parasitize tortoises.

The snake mite is an obligate, blood-sucking ectoparasite. It is a typical nidicole, spending most of its time in the dark, moist cracks and crevices of the cage and coming to the host only to feed (Camin, 1953). At 25°C the eggs incubate in less than 48 hours and the non-feeding stages, the larva and deutonymph, each have a duration of less than 24 hours. The mites feed only as protonymphs and adults and these stages will endure for one to five weeks with or without

a blood meal. However, the protonymphs are unable to molt into the deutonymphal stage and the adult females are incapable of producing eggs unless they have engorged on reptilian blood. Unmated females produce only male (haploid) eggs parthenogenetically, whereas mated females produce both male (haploid) and female (diploid) eggs in approximately equal numbers (Camin, 1953; Oliver, Camin & Jackson, 1963). The viability of all stages is adversely affected by humidities below the optimum of 95% R.H., but the effect is greater on the younger stages.

Directly or indirectly, O. natricis is an important cause, perhaps the primary cause, of snake mortality in zoos and other captive reptile collections around the world. Even light infestations will reduce the vigor and mar the appearance of captive reptiles. Infested snakes do not molt properly and often refuse to feed. Heavily parasitized snakes frequently die from exsanguination in a few weeks' time. In addition to such direct damage, these mites transmit some important reptile diseases, such as the highly virulent and often fatal hemorrhagic septicemia of snakes (Camin, 1948), caused by the bacterium Aeromonas hydrophila (Sanarelli, 1891). Although it is probably not the vector in nature, O. natricis is the primary vector of a haemogregarine disease of the lung and blood in captive snakes (Hull & Camin, 1960; Hull & Camin, in preparation) and there is suggestive evidence that this mite may also be capable of transmitting the filarial worm, Macdonaldius seetae Khanna, 1933, from snake to snake (Hull & Camin,

The primary goal of this investigation was to find an effective means of eradicating or control-

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ling the snake mite on captive reptiles. Previous methods had all proved inadequate, being either ineffective or impractical. Some killed too few mites. Others killed too many snakes. Our aim was to find a method suitable for routine application that would eradicate the mites without harming their hosts and, if possible, without necessitating the removal of the reptiles from exhibit. Methods requiring the individual dipping or dusting of reptiles are generally impractical, especially with large collections. Dusts and compounds using oils as vehicles tend to mar the appearance of the cages and the reptiles and therefore cause extra work in maintenance. Consequently, water soluble compounds and aqueous suspensions of acaricides would be the materials of choice, if they would also meet the other requirements.

During the course of our investigation we learned of the excellent results obtained in the control of the snake mite with Dri-Die 67 by Dr. I. Barry Tarshis in California. This work was subsequently published (Tarshis, 1960, 1961a, 1961b). Our results with this material were also promising, although not as promising as those of Dr. Tarshis, and other substances appeared to be somewhat superior. This will be further discussed.

It must be stated at the outset that time and funds did not permit us to carry this investigation to completion. However, because of the dramatic results obtained with two of the acaricides that were tested, even though our remaining data are not as clear-cut or as conclusive as might be desired, we feel that all of our results should be made available to those who may have use for them.

# MATERIALS AND METHODS

In order to develop a method of snake mite control that would be applicable under the widely varying conditions that prevail in different reptile collections, it was decided that tests should be conducted under optimal conditions for the mites and suboptimal or adverse conditions for the snakes. An acaricide that is effective under such conditions should then be effective in any situation that might be encountered in practice. For this reason initial tests for acaricidal activity were conducted against engorged adult female snake mites at 23-26°C and 80-95% R.H. Previous experience has shown that the engorged adult female is much more tolerant to variations in environmental conditions than any of the other active stages. Because of limitations of time and the fact that the incubation period of snake mite eggs is so brief, it was not practical or fruitful to test for ovicidal action. In addition, the egg is followed by the short-lived larval stage, which is the most vulnerable of all the active stages. Therefore, if an acaricide is active for at least 48 hours, it will kill the emerging larvae, even if it does not attack the egg.

Preliminary screening of 45 potentially acaricidal materials (Table I) was accomplished by treating the inner surfaces of 45 petri dishes, each with a different substance, and then placing ten engorged female mites in each dish. Active, immobilized and dead mites were counted after the first half hour, at approximately hourly intervals for the next 8 to 18 hours, at approximately 24 hours and at approximately 24-hour intervals thereafter until 96 to 168 hours had passed or until all the mites were dead. It was observed that some of the mites in the untreated controls were dead after four to five days and therefore mortality in the experimental dishes could not be reliably attributed to acaricidal action of the test compound after 96 hours. Among the 45 materials were several inert dusts and solvents that serve as vehicles for dust and spray toxicants. These served as treated controls against which the toxic materials could be compared. Some of these had mild acaricidal effects and were not used as vehicles for the test compounds.

After this preliminary screening, substances that had killed all of the mites in 24 hours or less were further tested for toxicity to reptiles. Materials that had caused 100% mite mortality in 48, 72 and 96 hours were noted and set aside to be tested further, if the more rapidly killing acaricides should all prove to be toxic to reptiles. Because small snakes were unobtainable in quantity at the time that we were ready to begin testing for herpeticidal action, American "chameleons" or anoles (Anolis carolinensis) were used. These are small, relatively fragile lizards, which are generally available from Florida at any time of the year. Later the same formulations were also tested against ring-necked snakes (Diadophis punctatus arnyi), which varied from 8 to 14 inches in length, and against young garter snakes (Thamnophis radix and Thamnophis sirtalis parietalis), which ranged in length from 18 to 26 inches. Such animals were utilized because previous experience had demonstrated that materials that are harmless to small, delicate snakes and lizards are generally also safe for use on larger reptiles. On the other hand, substances that have appeared non-toxic to large reptiles have frequently proved to be harmful and sometimes even lethal to smaller individuals.

Five anoles were used in each of the lizard tests. These were each given water prior to the

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.s. 2	Dosage Spec. <sup>4</sup> No. Dead/No. Tested 24hrs 96hrs 168hrs <sup>3</sup>		1	5/5 x	- (2	1	1	ı	1	1	1	1	1		1	1
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icity to	c.4 No.			0/5		1		1	}	1	1	1			1	ı
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	Dosag		1	2 g	i	I	1	1	1	1	I	1	ļ		1	1
	96hrs <sup>3</sup>		İ	×	1	I	×	I	1	1	9/10	2/10	0/10 (82)		2/10	1/10
	Tested 72hrs		İ	×	1	1	×	1	1	ı	7/10	1/10	0/10		1/10	0/10
Toxicity to Mites i	No. Dead/No. Tested 24hrs 48hrs 72hrs		6/10 (41).	×	$\frac{3}{10}$	1	×	1	1	3/10 (28)	7/10	0/10.	0/10		0/10	0/10
cicity to	No. Dea		3/10.	10/10	2/10 2/10		×	6/10	1	3/10	3/10	0/10	0/10		0/10	0/10
To	4hrs 2		0/10	7/10 1	0/10	1	(1)	3/10.	1	0/10	0/10	0/10	0/10		0/10	0/10
	Dosage		5 drops	3 mg.	.3 mg.	1	3 mg: 1	3 mg.	ı	3 mg.	5 drops	5 drops	5 drops		20 mg.	20 mg.
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	Dilution		1:48 by vol. ·	None	1:10 by wt.	I	None	5%	I	1:800 by wt	1 ml/gal	9 g/gal	4 g/gal		3%	3%.
	Use Form		Oil Solution	Dust	Dust w/Attaclay X-250	ĺ	Dust:	Dust w/Sulphenone $50 \mathrm{W}^7$	1	Dust w/Attaclay X-250	H <sub>2</sub> O Emulsion	H <sub>2</sub> O Suspension			Dust	Dust
Manufacturer	· or Formulator		Fairfield (FMC)	Fairfield (FMC)		***************************************	Hercules	Union Carbide	Hooker	Geigy	Acme Paint	Rohm & Haas			Chemagro	Chemagro
Product	(Alphabetical by Classification)	ORGANIC CHEMICAL PRODUCTS  Botanicals:	Pyrethrins, Synergized of Pyrethrins, Synergized Syrenone Roach Spray Concentrate 1.5% Pyrethrins 7.5% Piperonyl Butoxide	Pyrenone Dust Base, BP-13-30	6.0% Piperonyl Butoxide	Rotenone6	Hercules 5727, Tech. N-methyl m-isopropylphenyl carbamate	Sevin, Tech.	Chlorinated Aryl Hydrocarbons: Lindane <sup>8</sup> DDT Relatives:	Chlorobenzilate 25W 25% Wettable Powder	Dimite 25% Emulsifiable Conc.	Kelthane W 18,5% Wettable Powder		Heterocyclic Compound (not listed elsewhere):	Bayer 30686 2,3-quinoxalinedihiol cyclic trithiocarbonate	Nitrophenyl Compound: Bayer 28589 2,6-di-len-butyl-4-nitrophenol

TABLE I, COMPARATIVE ACARICIDAL ACTIVITY AND TOXICITY TO REPTILES OF VARIOUS PRODUCTS SCREENED

Product	Manufacturer				Toxic	Toxicity to Mites 1	-		L	Toxicity to Reptiles 2	to Repti	es 2	
(Alphabetical by, Classification)	or Formulator	Use Form	Dilution	Dosage	No 4hrs 241	No. Dead/No. Tested 24hrs 48hrs 72hrs	1 1	D 96hrs <sup>3</sup>	Dosage Spec. <sup>4</sup> No. Dead/No. Tested 24hrs 96hrs 168hrs	pec.4 N	No. Dead 24hrs 90	ad/No. Te 96hrs 16	Tested 168hrs <sup>3</sup>
ORGANIC CHEMICAL PRODUCTS Phosphorus Containing Compounds— Phosphorous Aliphatic Derivatives:													
Experimental Insecticide 18706	Amer. Cyanamid	H <sub>2</sub> O Emulsion	4 ml./gal.	5 drops	8/10 10/10	10 ×	×	×	1	1	1	1	
0,0-dimethyl-S-(N-ethyl carbamoyl methyl) phosphorodithioate			2 ml./gal.	5 drops	8/50 41/	41/50 50/50 (45)	×	×	5 ml,	RS	0/10	0/10	0/10
DDVP (Vapona)	Shell	H <sub>2</sub> O Emulsion	0.2%	g drops	10/10	×	×	×	2 ml.	ı	4/5	5/5 (66)	×
Dibrom 8E 8#/gal: Emulsifiable Conc.	California Chem,	$ m H_{2}O$ Emulsion	4 ml./gal.	5 drops	0,	10/10 x (22)	×	×	2 ml.	ı	0/5		0/5
			4 ml./gal. 2 ml./gal.	5 drops	4/50 23/	23/50 36/50	39/50	45/50	5 ml. 5 ml.	RS RS	0/5 0/10	1/5 0/10	$\frac{1}{5}$
Dylox 50% Soluble Powder	Chemagro	H <sub>2</sub> O Solution	2 g./gal.	5 drops	5/10 8/	8/10 10/10 (26)	×	. <b>×</b>	1	-	1	ı	1
Malathion E-5 5#/0al: Fimulsifiable Conc.	Thompson-	H <sub>2</sub> O Emulsion	6 ml./gal.	$5  ext{ drops}$	2/10 10/	10/10 · x	×	×	2 ml.	ы	0/2	0/5	0/2
			6 ml./gal.		1		I	I	5 ml.	RS.	5/5	×	×
			2 ml./gal.	I			1	İ	5 ml.	CS		2/10	2/10
Systox Spray Concentrate 4#/gal.; Emulsifiable Conc. Phosphorous Hetrocyclic Derivatives:	Chemagro	H <sub>2</sub> O Emulsion	5 ml./gal.	5 drops	2/10 5/	5/10 6/10 (33)	1	1	1	1	1	i	ı
Co-Rai 25W 25% Wettable Powder	Chemagro	H <sub>2</sub> O Suspension	2 g./gal.	5 drops	0/10 4/	4/10 4/10	4/10	7/10	1		ı	ı	1
Co-Ral Livestock Duster	Chemagro	Dust	0.5%	3 mg.	0/10 4/	4/10 7/10 (33)	I	1	1	1	1	1	1
Delnav. 47% Emulsifiable Conc.	Hercules	H <sub>2</sub> O Emulsion	1:300 by vol.	5 drops	0/10 6/10		x 19/50	x 21/50	1 1	1 1	i 1	1 1	1 [
Diazinon 20S. 20% Oil Soluble Concentrate	Geigy	Oil Solution	1:192 by vol.	5 drops	0/10 5/	-	- 1		1	1	1	ı	ı
Diazinon 25E 25% Emulsifiable Conc.	Geigy	$ m H_2O$ Emulsion	.1:240 by vol. 1:240 by vol. 1:480 by vol.	5 drops	3/10 10/	10/10. x 11/50 17/50	× 31/50	× 36/50	2 ml, 5 ml, 5 ml,	L RS RS	0/5 0/5 0/10	0/5 1/5 0/10	0/5 2/5 0/10
Guthion 25W 25% Wettable Powder	Chemagro	H <sub>2</sub> O Suspension	2 g./gal.	5 drops		0/10 0/10.	. 0/10	0/10 (82)	I	ı	1	1	ę.

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Droging	Manufacturer				Tox	Toxicity to Mites 1.	Mites 1.		-		Toxicity to Reptiles 2	to Repti	les 2	
(Alphabetical by Classification)	Formulator	Usé Form	Dilution	'Dosage	r 4hrs 2	No. Dead/No. Tested 24hrs 48hrs 72hrs	ad/No. T 48hrs 7		96hrs <sup>3</sup>	Dosage	Spec. <sup>4</sup> No. Dead/No. Tested 24hrs 96hrs 168hrs	No. Dead/No. Tested 24hrs 96hrs 168hrs <sup>3</sup>	/No. T	ested 38hrs <sup>3</sup>
ORGANJC CHEMICAL PRODUCTS Phosphorous Phenyl (carbocyclic) Derivatives: Dicapthon, Tech. Methyl Trithion, Tech. Trithion, Tech. Stauff	tvatives: Amer. Cyanamid Stauffer Stauffer	Ethyl Alcohol Sol. Ethyl Alcohol Sol. Ethyl Alcohol Sol.	1:1000 by wt 1:1000 by vol. 1:1000 by vol.	5 drops 5 drops 5 drops	0/10 0/10 0/10	4/10 0/10 0/10	5/10 0/10 0/10	6/10 5/10 1 5/10 1	7/10 10/10 10/10	111	111		111	111
repetents: Benzyl benzoate Tabutrex, Tech.	Monsanto Glenn Chemical	Ethyl Alcohol Sol. Ethyl Alcohol Sol.	5% 3 ml./gal.	5 drops 5 drops	2/10 5/10	3/10 6/10	8/10 (41)	1-1	1 1	1.1	1 1	1 1	11	11
Delphene, Tech.	Hercules	Ethyl Alcohol Sol.	5%	5 drops	5/10 1	10/10 (19)	×	×	×	2 ml.	ī	5/5 (3)	×	×
			2%	1	1	1		1	]	5 ml.	RS	5/5 (1)	×	×
Rodenticide: Warfarin	Penick	Dust w/Attaclay X-250	0.025%	3 mg.	2/10	7/10	8/10	1	-	0.5 g.	님	0/5	0/5	9/2
			0.025%	1	1	١	1	1	١	2 g.	RS	0/5	1/5	3/5
Sulfonates, Sulfides, Sulfones: Mitox 40W 40% Wetable Powder	California Chem.	H <sub>2</sub> O Suspension	4.5 g./gal.	5 drops	0/10	0/10	1/10	. 5/10	6/10 (82)	I	1	1	1	1
Orthotran 50W9	California Chem.	H <sub>2</sub> O Suspension	4.5 g./gal.	5 drops	0/10	1/10	1/10	1/10	2/10	1	I	1	ı	ı
Ovotran W9 50% Wettable Dowder	Dow	H <sub>2</sub> O Suspension	3 g./gal.	5 drops	0/10	6/10	ı	i	]	1	1	1	1	ı
Sulphenone 50W 50% Wettable Powder	Stauffer	H <sub>2</sub> O Suspension Dust	3 g./gal. None	5 drops	0/10	0/10	11	1.1	11	5 ml. 2 g.	GS GS	0/2 0/2	0/2 1/2	0/2 2/2 139)
Tedion 25W 25% Wettable Powder	Niagara (FMC)	H <sub>2</sub> O Suspension	4.5 g./gal.	5 drops	0/10	0/10	3/10	5/10	5/10	1	I	1	1	]
Other Organic Chemical Products: Ethyl Alcohol FOAH Modified 1% Sodium borohydride in	Missouri Solvents Metal Hydrides, Inc.	Liquid H <sub>2</sub> 0 Solution	95% 10,000 ppm	5 drops	0/10 0/10	0/10	3/10 (28)	11	1.1	11	1 1	11	11	ļ 1
Methyl salicylate 10 (Oil of wintergreen)		Liquid	None	5 drops	5/10 10/10 (21).	10/10 (21)	×	×	×	1	1	1	1	-

TABLE I. COMPARATIVE ACARICIDAL ACTIVITY AND TOXICITY TO REPTILES OF VARIOUS PRODUCTS SCREENED

								-					
Product	Manufacturer				Toxic	Toxicity to Mites 1	3.1		T	oxicity to	Toxicity to Reptiles 2	2	
(Alphabetical by Classification)	or Formulator	Use Form	Dilution	Dosage	No 4hrs 241	No. Dead/No. Tested 24hrs 48hrs 72hrs		D 96hrs3	Dosage S	pec. <sup>4</sup> No	Dead/N. Dead/N.	Spec. <sup>4</sup> No. Dead/No. Tested 24hrs 96hrs 168hrs <sup>3</sup>	
INORGANIC CHEMICAL PRODUCTS Silica Aerosels and Derivatives													
Dri-Die	Davidson Chem.	Dust	None	3 mg.	0/10 8/	8/10 10/10	×	×	63	RS 1	1/5 3/5	5 4/5	
(50-67) 4% Ammonium fluorosilicate	National Biocides		None	20 mg.	.16/50 48/50	(30) 50 50/50	×	×	1	ľ	1	1	
			None	10 mg.	21/50 50/50	50 ×	×	×	1			1	
Silikil 4% Ammonium fluorosilicate	United Heckathorn	Dust	None	3 mg.	0/10 2/	2/10 8/10 (28)	ŀ	1	ŀ	· 1	1	1	
Silikil D A dense grade of the above.	United Heckathorn	Dust	None	3 mg.	0/10 7/	7/10 9/10 (28)	١	ł	1	i	1	1	
Silikii PY <sup>11</sup> 1.5% Ammonium flurosilicate 0.11% Pyrethrins	United Heckathorn	Dust	None	3 mg.	7/10 10/10 (17)		×	×	1	,	1		
Tales:													
Attaclay	Min. & Chem. Corp. of America	. Dust	None	3 mg.	0/10 6/	6/10 10/10 (28)	×	×	1		1	1	
Attaclay X-250	Min. & Chem., Corp. of America	. Dust	None	3 mg.	0/10 0/	0/10 0/10 (28)	I		т ф	RS 0	0/10 0/10	.0 0/10	0
PROPRIETARY PRODUCTS				;									
<ul> <li>Baume Bengue</li> <li>(contains Methyl salicylate <sup>12</sup>,</li> </ul>	Thos. Leeming & Co.	o. Salve	None	Vapor from	0/10 10/10	10 ×	×	×	23 89	GS C	2/2 (2)	Ħ	
menthol and lanolin)				3 mg.	1	1	1	1	2 99	RS 2	2/2 x 9 minutes	×	
El Vampiro Flea Powder	J. Strickland & Co.,	, Dust	None	3 mg.	0/10 6/	6/10 10/10	ĸ	×	2 g.	RS 5	5/5 x	×	
0.125% Pyrethrins 0.625% Rotenone	ivacinguis, i cinii.		None	ı	1	(c*)  -	.1	ŀ	2 is	GS 22	(5) 2/2 x (18)	×	
1.250% other Cubé extractives Wonder Mite Ball <sup>13</sup>	Pet Accessories, Inc.	. Vapor	None	1	1/10 1/	1/10 3/10 <sup>14</sup>	14 —	1	1	' ;		1	

1All controls lived for at least 96 hours. 2 In tests using 5 ring-necked snakes (RS), one of the 5 controls died after 168 hours. 3 The number of hours elapsed at the termination of the test is shown in parentheses when this number is smaller than that shown at the top of the column. — means "no further data or observations"; x means "all animals dead". 4 Under species of repilies, L—llzard (Anolis carlinensis); RS—ring-necked snake (Diadophis purcleutus arryi); and GS—garter snake (Thomnophis radix or Thannophis siradis parietalis). 5 Also see Silikil PY under Inorganic Chemical Products and El Vampiro Flea

Powder under Proprietary Products, both of which contain pyrethrins. 6 See El Vampiro Flea Powder under Proprietary Products. 7 Also see Sulphenone 50W under Sulfonates, etc., in the Organic Chemical Products. 8 See "Wonder Mite Ball" under Proprietary Products. 9 A brand of Ovex. 10 A principal ingredient in Baume Bengref, see Proprietary Products. 11 Also see Pyrethrins, Synergized, under Botanicals in Organic Chemical Products. 12 Also see Methyl salicylate under Other Organic Chemical Products. 13 Net contents 14 A.o. in plastic ball. Contained 3.5% Lindane and 4% other isomers of Benzene hexachlorde. 14 One of the mites killed was apparently crushed by the ball.

test and were placed in one-quart glass jars. The acaricides were then applied at the rate of 2 cc. for spray compounds and 0.5 gram for the one dust compound tested against lizards. A moist cloth was secured over the opening of each jar to prevent escape of the lizards, to permit air circulation and to help maintain a reasonably high humidity. Observations were made at one to four hour intervals over the first 100 hours, with gaps of 12 to 15 hours between hours 6 and 18, 32 and 44, 52 and 66 and 75 and 90. Thereafter, checks were made at approximately 24-hour intervals until 168 hours had passed. At each check, the numbers of healthy, affected and dead lizards were recorded.

The first tests on snakes were conducted in essentially the same manner, using groups of five ring-necked snakes and applying the acaricides at the rate of 5 cc. for sprays and 2 grams for dusts. However, this proved to be too drastic, especially with the dust compounds, and several snakes died by strangulation, even in the controls. These were found with their mouths and throats full of the fine, dry dusts. In subsequent tests, one-gallon, wide-mouthed glass jars were used and drinking water was supplied in each jar. Acaricides were applied in the same quantities as before and the tests were conducted on groups of 10 ring-necked snakes per jar and two garter snakes per jar because the latter were in shorter supply. In these experiments observations were made at intervals similar to those used in the lizard tests and all of the snakes survived unharmed in the controls for more than 168 hours. All of the acaricides in the aforementioned tests were applied in the concentrations suggested by their manufacturers or suppliers.

Materials that passed the first two tests, killing samples of 10 mites in less than 24 hours while proving to be harmless to reptiles, were further tested against the mites. In this group of tests the acaricides were applied at one-half the recommended concentration and 50 mites were used in each test. Following this series, four of the more promising acaricides were selected for testing under simulated zoo conditions.

Six small and three larger reptile display cages were obtained from the Swope Park Zoological Gardens in Kansas City. These were constructed of wood, with a screen top and a glass front. The inner two side walls and back were lined with galvanized sheet metal upon which was painted a "natural habitat" scene. The smaller cages measured 24 inches in width, 16 inches in depth, and 11 inches in height. The larger cages were 17 inches wide, 22 inches deep and 29

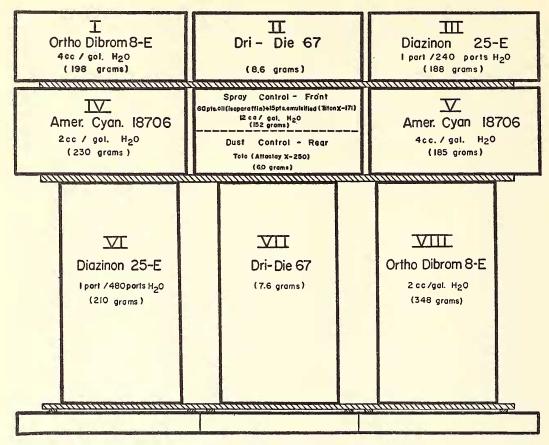
inches high. The floor of each cage was covered with a mixture of vermiculite and white sand at the rate of approximately 12 to 13 pounds for the small cages and 16 to 17 pounds for the larger ones. Each cage was provided with a water bowl and several natural objects, such as rocks and small tree branches.

One of the smaller cages, which was to serve as the control cage, was divided into front and rear halves by a partition so that both liquid spray and dust controls could be employed.

The cages were arranged in three tiers of three cages each, with the larger cages on the bottom row over some large pans containing water. The water served to raise the humidity in the immediate vicinity of the cages and to prevent the escape of the mites. The control cage was placed in the middle of the second row, in the center of the battery of nine cages (Text-fig. 1). Temperature was maintained at approximately 25° C throughout the experiment but we were unable to maintain constant humidity. Ambient humidity ranged from 48 to 74% R.H., but humidities within the cages and in the microhabitats were probably considerably higher.

Eighty-seven snakes, representing five species, were used in this experiment. These were distributed as evenly as possible, according to size and species, among the cages (Table II). The snakes that were obtained from commercial dealers and from the Swope Park Zoo were already infested with mites. The remaining freshly collected snakes became infested soon after being placed in the colony. All of the snakes appeared to be in good health and most of them were feeding well. All were offered food (mice, earthworms and raw fish) before the start of the experiment. A few refused all food, but no attempt was made to force-feed them. At the start of the experiment all but the smallest of the snakes were well infested with snake mites, but the mite population varied in each cage. Very small snakes do not support significant numbers of mites because their scales are small and overlap closely. Therefore the juvenile garter snakes and the small ring-necked snakes were used to test the safety of the treatment rather than its effectiveness in mite eradication.

Each of four materials was tested at two dosage levels, with each dosage level being tested in a separate cage (Text-fig. 1). The cages were treated individually in another laboratory in order to avoid contamination of any of the other cages in the battery. Liquid compounds were applied with a portable commercial stainless steel pressure sprayer (B & G Model 54-S) and dusts were applied by means of a commercial



Text-fig 1. Arrangement of cages for "field" testing under simulated zoo conditions.

hand-operated insecticide duster (Getz Duster). The equipment was thoroughly washed and dried before each use. The materials were applied with steady, even strokes. First, the inside walls were sprayed (or dusted) from bottom to top, next the top and the floor of the cage were treated and, finally, the acaricide (or control material) was applied to the outside walls of the cage. Every exposed area was covered with the material and cracks, corners, natural objects and other areas in which mites tended to congregate were liberally treated in order to assure that the acaricide penetrated these places. Immediately before the application of the test substances, fresh water was placed in each cage. The formulation was applied around, on and in the water bowl. Snakes were not removed during treatment and were sprayed liberally as the floor of the cage was treated. None of the snakes were handled during the application of the materials.

After the initial treatment the test was allowed to run for eight days. During this time the snakes were given fresh water daily, but none were fed. Observations were made at the start of the experiment and at approximately 24-hour intervals for the first 96 hours and again at 168 and 192 hours. The total operation averaged twelve hours per day.

At the beginning of each observation period each cage and its contents were inspected with aid of a hand lens and a restricted beam flashlight. The snakes were checked but were not handled. Notations were then made as to the apparent presence or absence of a mite infestation and rough estimates as to whether infestations were light, moderate or heavy were made.

Next, a 5 ml. sample of sand was taken from the center and from each of the four corners of the cage. Because in each cage the water bowl occupied one corner and natural objects, such as rocks and branches, occupied another, two of the corner samples in each cage were taken near these. The five sand samples were mixed together and from the mixture a 1 ml. sample was taken for microscopic analysis. This sub-sample was spread out in a 50 mm. plastic petri dish and living and dead mites were counted.

Finally, each snake except the juvenile garter snakes and the ring-necked snakes was thoroughly examined for mites. It was not feasible to actually count every mite on the body of each snake, so a method was used that would permit comparative estimates to be made. The snake was held over a white cloth with one hand of the observer placed immediately behind the head of the snake. The other hand was then passed slowly along the length of the snake from the anterior to the posterior end. Many of the mites would cling to the hands, but most of them dropped onto the cloth. With the help of an assistant, the mites on the hands and on the cloth were counted and recorded. The anal region, chin shields and eye sockets were also checked for mites and the snake was then quickly returned to its cage. The counts were neither exact nor complete, but they provided a reliable means for comparing mite populations in the different cages. After sampling and counting, the mites were placed back into the cage from which they had come in order to reduce the effect that the sampling technique might have on the course of the experiment.

At each observation period the activities and apparent condition of the snakes were also noted.

#### RESULTS

Of the 45 materials that were tested initially against samples of ten engorged female mites each, eleven killed all of the mites in less than 24 hours (Table I). One of these, Baume Bengué ointment, had been recommended as a potential acaricide because it had been used effectively,

due to its repellent action, in confining mites in various laboratory experiments (Strandtmann & Wharton, 1958). The active ingredient in this product is methyl salicylate or "oil of wintergreen," so this was also tested and proved highly effective against the mites. However, when the Bengué ointment was applied by hand to two ring-necked snakes and two garter snakes, these immediately began to writhe and thrash about violently. The two ring-necked snakes were dead in less than ten minutes and the garter snakes died within two hours. Another of the eleven candidates, Silikil PY, is principally a silica aerogel similar to Dri-Die 67, the compound with which Tarshis (op. cit.) has obtained such encouraging results. In our initial tests, Silikil PY appeared to be somewhat superior to Dri-Die 67. Silikil PY killed ten mites in 18 hours, while Dri-Die 67 took 30 hours to kill all ten. However, because two other Silikil formulations were less effective than Dri-Die and because of the results obtained by Tarshis with this latter product, we decided to confine further testing of silica aerogels to Dri-Die 67. Nevertheless, Silikil PY showed promise and should be tested more thoroughly. It is interesting to note that most of the remaining formulations among the "most promising 11" are organic phosphorus-containing acaricides (Table I).

Because of the limited numbers of small reptiles available for testing, one other acaricide (Hercules 5727) that had killed ten mites in less than 24 hours and Dylox, which had killed ten mites in 26 hours, were not screened for toxicity

TABLE II: DISTRIBUTION OF SNAKES FOR ACARICIDE TEST UNDER SIMULATED ZOO CONDITIONS.

			Snakes		
Cage	Elaphe obsoleta obsoleta	Natrix sipedon sipedon	Thamnophis sauritus sackeni	Thamnophis sirtalis parietalis	Diadophis punctatus arnyi
I	1 L*	1 M*, 1 S*	2 M	2 J*	none
II	1 L	1 M, 2 S	2 M	1 S, 3 J	none
III	1 L	2 M	2 M	1 S, 2 J	none
IV	1 L	1 M, 2 S	2 M	1 M, 2 J	none
V	1 L	1 M, 2 S	2 M	2 J	none
VI	1 L	3 M	2 M	2 J	none
VII	1 L	1 M, 2 S	1 M, 1 S	2 Ј	1 S
VIII	1 L	2 M	1 M, 1 S	2 S, 2 J	none
Spray			,		
control Dust	1 L	1 M, 2 S	2 M	1 M, 2 J	none
control	1 L	1 M, 2 S	2 M	1 S, 2 J	1 S

<sup>\*</sup>L = Large = more than 36 inches in length.

M = Medium = 24 to 36 inches.

S = Small = 12 to 24 inches.

J = Juvenile = less than 12 inches.

to reptiles. The remaining seven materials were tested against small lizards and snakes according to the procedures previously described.

Delphene (diethyltoluamide), DDVP and Pyrenone Dust Base all proved to be highly toxic to anoles and ring-necked snakes. Malathion appeared to be safe for the lizards, but killed ringnecked snakes. This compound had been used effectively in snake mite control several years earlier at the Lincoln Park Zoo in Chicago (Camin, unpublished). At that time it was found that dosages as low as 0.01% Malathion emulsifiable in water was effective against the mites and harmless to snakes, if it did not come into contact with the mucous membranes of the latter. However, if even a few drops got into the mouth of a snake, the animal died in a few hours. This was confirmed in the present tests. Adverse effects of Malathion on other vertebrates are not unknown. In commercial production of laboratory mice near Kansas City, it was found that 5% or 2½% Malathion dusts applied to the bedding completely controlled the mouse mite, Mycoptes musculinus. Simultaneously, production of marketable young mice was very noticeably reduced in Malathion-treated populations when compared with that of the untreated miteinfested mice (Beran, Cornett & Shuyler, unpublished). Therefore, inasmuch as equally effective and safer materials are now available, Malathion can no longer be recommended for snake mite control.

Of the eleven candidates that killed ten mites in less than 24 hours, only three proved to be harmless to the lizards and small snakes. These were American Cyanamid 18706, Ortho Dibrom 8-E and Diazinon 25-E. It should be pointed out that Hercules 5727 and Dylox also remain as potentially useful compounds in the control of the snake mite, at least until their effects on snakes are known. These should be tested for toxicity to reptiles.

In the initial tests, four additional compounds killed all ten mites in 30-35 hours. These were Delnav, El Vampiro Flea Powder, Dri-Die 67 and one of the formulations of Attaclay. Delnav is another of the organic phosphorus-containing compounds and should be further tested for toxicity to reptiles. El Vampiro Flea Powder, a mixture of piperonyl cyclonene, pyrethrins and rotenone, was recommended to us by an amateur herpetologist who has used this product for several years with good results and no apparent harm to the snakes. We found it to be effective against the mites, but highly toxic to small snakes. Attaclay, a tale that is used as a diluent for some of the dust formulations, probably kills mites by desiccation in much the same manner as the silica aerogels and is probably deserving of further attention. Attaclay X-250, another form of this talc product, killed no mites in the initial tests.

Dri-Die 67, principally a silica aerogel that abrades the epicuticle and adsorbs its lipid waterproofing layer, thus causing death by desiccation, has given somewhat erratic results. Because this compound is not in itself thought to be toxic to the mites, but kills indirectly through desiccation, its effectiveness is probably affected in some degree by the humidity to which the mites are subjected and also by the activity of the mites. In the initial test against ten mites, 3 mg. of Dri-Die 67 killed half of the mites in 20 hours and required 30 hours to kill all ten. In two subsequent tests, using 10 and 20 mg. of Dri-Die 67 against samples of 50 engorged female mites each, the lower concentration killed all the mites in nine hours while the higher concentration required 27 hours to produce 100% mortality. In both cases half the mites were dead after only five hours. While employing Dri-Die 67 for snake mite control in the laboratory at The University of Kansas it had been noted (Camin, unpublished) that while the compound produced rapid desiccation and death in mites that were off the host or crawling about freely on the host, mites that were attached under the scales and feeding appeared to remain attached much longer than usual. While being affected by the acaricide, these mites apparently compensated for the body water lost through dessication by imbibing more blood from the host. More than a week after treatment with Dri-Die 67, although no mites could be found crawling freely about the cage or on the host, fully engorged and living mites were still plentiful under the scales and in the eye sockets of the hosts. It was also noted that while Dri-Die 67 appeared to be harmless to large snakes and even to most small snakes, it apparently caused some desiccation in the reptiles too, because treated snakes drank much more water than did untreated snakes in nearby cages. Generally, the snakes seemed to be able to maintain their water balance and suffered no harm, but some of the smaller snakes died after being treated with Dri-Die 67, apparently due to desiccation. However, because of the excellent results reported by Tarshis (op. cit.) and the promising, though erratic results that we had obtained with Dri-Die 67, we decided to continue to test this product.

Sulphenone and Warfarin were also recommended to us as potentially effective acaricides. Sulphenone, in aqueous suspension, was harmless to snakes, but it also killed no mites. The producers then suggested that we use it dry and undiluted. This proved to be toxic to snakes and no

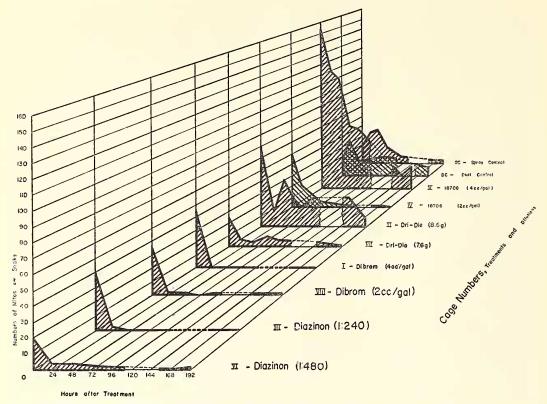
further tests against mites were conducted. Warfarin, a rodenticide, killed more than half the mites in 48 hours, but failed to kill any more. Further tests showed it to be detrimental to small snakes, so testing was discontinued. Several other materials produced significant mite mortality (Table I), but time did not permit us to test them for toxicity to reptiles.

Dri-Die 67 and three other materials were selected for final "field" testing on mite-infested snakes under simulated zoo conditions. The three candidates, all of which had killed 10 mites in 24 hours and had proved relatively non-toxic to small reptiles, were American Cyanamid 18706, Ortho Dibrom 8-E and Diazinon 25-E. As was done earlier with Dri-Die 67, each of the three formulations was tested at one-half concentration against samples of 50 engorged female mites prior to the "field" test. All produced 90-100% mortality, but acted more slowly than they had at full concentration. American Cyanamid 18706 killed more than half the mites in less than 20 hours. At 24 hours none of the mites were active, but nine were still alive. At 44 hours 100% mortality was achieved. Ortho Dibrom 8-E had killed half of the mites by the end of 30 hours and of the remaining 25, only five were able to move about. By the end of 96 hours only five were still alive and four of these were paralyzed, but two remained alive to the end of the test at 168 hours. Diazinon 25-E required approximately 60 hours to kill half the mites and at 96 hours, the time at which a few of the mites in the controls began to die, fourteen were still living. Four mites survived to the end of the test, but none were moving at that time.

The final experiment, simulating conditions to be found in some zoo collections of reptiles, was purposely designed to make the eradication of snake mites difficult. We believed that if eradication could be accomplished under such circumstances, we would then have a method of control that could be expected to meet with reasonable success even in the most poorly curated of serpentaria. This is most important because such establishments are often the sources of specimens for the most carefully kept collections. Therefore, the control cages were placed in such a manner as to give the mites from these cages free access to the treated cages. It was originally intended that this experiment would be repeated several times with different arrangements of the cages that were treated with the various acaricides, in order that the experiment would not be biased for or against any of the test materials. Unfortunately, time and funds did not permit the repetition of these experiments and the project had to be terminated without being truly completed. Nevertheless, keeping these drawbacks in mind, much valuable data resulted from the one "field" test that was completed.

In an evaluation of the data from the "field" test, several things must be taken into account. Because the lower cages were continuously subjected to re-infestation by mites dropping from the cages above, no two cages in the series were actually subjected to identical conditions. The effects of mites (primarily engorged mites) dropping from the upper to the lower cages were partly counter-balanced by the tendency of unfed mites to climb upwards, thus resulting in some movement of mites from the lower to the upper cages. Considering only downward and upward migration of the mites, then the infestation in cage VII, being directly under the control cages, would be the most difficult to combat. Cages VI and VIII would rank next, cages IV and V would be third, cage II would be fourth, and cages I and III would be fifth or the easiest to control. Another factor to be considered, however, with regard to the probability of re-infestation, is the amount of surface exposed directly to the control cages. From this standpoint cages II, IV, V and VII would be in the worst positions, while cages I, III, VI and VIII would be the most favored. In addition to the problem of re-infestation, the initial mite population size must be taken into account. Because of the facts that populations increase geometrically and the snake mite can complete its life cycle in a very short period of time (13 to 19 days at 25° C), the size of the initial infestation is of paramount importance (Text-fig. 2). From the standpoint of this factor alone, the populations in cages I, III, VIII and the control cages were approximately equivalent. The population in cage VII was lower and that of cage VI was considerably lower. Cages II and IV were more heavily infested and the infestation in cage V was extremely heavy. Taking all these factors into consideration, but giving more weight to the factor of the size of the initial infestation, the mites in cage V should be the most difficult to eradicate and, in order of decreasing difficulty of control, the remaining cages would rank as follows: cage II-2nd; cages IV and VII-3rd; cage VIII-4th; and cages I, III and VI-5th (Text-fig. 1).

The migrating habits of the mites were considered in the placement of each treatment with respect to the spray-dust control cage. We were aware of other promising work with Dri-Die, so we thought it best to give this material a critical test with respect to mite control by placing it above and below the control cages. Less was known about the properties of American Cy-



Text-Fig. 2. Comparative effects of four acaricides, at two concentrations each, on snake mite infestations. (No observations were made at 120 or 144 hours).

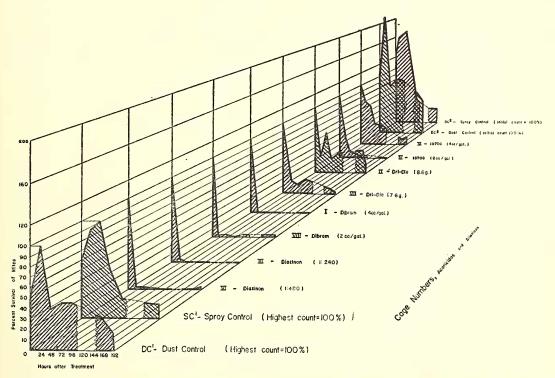
anamid 18706; therefore, it was thought best to test this material in a placement that should be average with respect to migration. In order to test Diazinon, Dibrom and Dri-Die more critically, the lower concentrations were used on the bottom row of cages and heavier concentrations on the upper row of cages.

Merely by random chance, the three lower cages had the lightest infestations of mites, the two upper corner cages had moderate infestations, as did the cage to the left of the control cage, while the cage above the control cage and especially the cage to the right of the control cage had much heavier infestations.

At first examination, it would appear that all four candidates, at all eight concentrations, were highly effective. Judging by means of the method this is commonly used to evaluate the magnitude of the mite problem in reptile collections, that of observing whether or not there are mites crawling freely about on the surfaces of the snakes or on the walls and objects in the cage, all treatments were apparently completely successful. In the control cages, mites were readily observable throughout the experiment. In those cages treated with American Cyanamid 18706 (cages

IV and V) mites were visible for 72 and 96 hours, respectively, but then disappeared. In cage VI, treated with Diazinon 25-E, mites could no longer be seen after 48 hours, while in all other treated cages the mites were no longer apparent after only 24 hours, with the exception of their temporary reappearance in cage II at 72 hours. Although this method is perhaps reliable in detecting heavy infestations, the fact that it should not be too heavily relied upon is attested to by the remaining data. The sampling of living and dead mites in the sand of the cage is also of some value, but it proved to be too erratic to be very accurate. The only completely reliable method of estimating mite populations on snakes is actually to count the mites on various parts of the body of the snake with the aid of a microscope. This, of course, is not feasible. However, a good indication of the extent of the mite problem can be obtained by sampling the mite populations that are on the snakes by removing them and counting them with the naked eye. It must be noted that the method of sampling that we used, while giving a fairly accurate estimate of the relative sizes of the mite populations, does not yield completely accurate estimates of the absolute population sizes. This method samples only the mites that are crawling about on the snake, not those that are attached and feeding. Therefore, population estimates are more accurate for the cages that were treated with substances that disturbed the mites and caused them to stop feeding and move about. Thus, the populations of mites in the control cages apparently rose at first because the mites were disturbed by the control treatments even though they were not killed by them. Similar disturbance probably occurred in the other eight cages, too. However, in the latter, most of the activated mites, coming in contact with freshly applied acaricide, probably died. Thus, although an initial apparent rise in population probably also occurred in these cages immediately following treatment, the population had decreased significantly by the time 24 hours had passed. It should be noted, however, that although in several cases the experimental treatments appeared to be 100 percent effective, this does not necessarily indicate complete eradication because some mites may have remained under the scales of the hosts, attached and feeding. These would not have been sampled and, in the case of acaricides with little residual activity, these mites could eventually re-establish the infestation.

After sampling and counting the mites from each cage, the mites were placed back into the cage from which they had been removed. It was hoped that this procedure would minimize any direct influence that the sampling technique might have on the course of the experiment. Unfortunately, as our data from the control cages revealed, the sampling was, in itself, fairly effective in reducing the mite populations on the snakes. This fact must be taken into account when evaluating the effects of the various treatments. The sampling technique reduced the populations of mites by approximately 50% in 48 to 72 hours in both controls. If, for purposes of comparison, the highest count obtained is regarded as 100%, then both control populations were reduced to less than 20% by the end of eight days (Text-fig. 3). On the other hand, if it is assumed that the acaricide-treated mite populations would also have shown an initial apparent rise, if mites had not been killed by the treatment, then it is probably more accurate to compare initial samples of acaricide-treated populations with initial control populations. In so doing, the initial count is regarded as 100% and subsequent counts are calculated as percentages of the initial estimate (Text-fig. 3). In this case, the mite



TEXT-FIG. 3. Effects of four acaricides, at two concentrations each, compared by percent survival of mites. (No observations were made at 120 or 144 hours).

populations never fell below 35% in the dust control nor below 25% in the spray control.

American Cyanamid 18706 greatly reduced the mite populations, but its action appeared to be much slower than that of other materials. It did not completely eradicate the mites and those mites that survived the initial application in cage V still caused considerable damage to the snakes. It must be remembered, however, that the cages treated with 18706 (cages IV and V) contained two of the most challenging infestations (Textfig. 2). If other promising substances did not appear to be available, this acaricide would be well worth using and, therefore, deserves further testing. The fact that better control was accomplished with the lower concentration (cage IV) than with the higher (cage V) emphasizes the importance of considering the initial population size when evaluating the results. One snake died in cage IV and three died in cage V. Because of the fact that the mite populations were extremely high and because the juvenile snakes in both cages survived the experiment, it is suspected that the snakes may have died from exsanguination by the mites rather than the effects of the pesticide. From this experiment alone the possibility of toxic effects on the snakes cannot be completely excluded. However, previous tests had shown this compound to be harmless to small snakes.

One snake in the "Spray Control" cage also died during the course of the experiment. This specimen, too, appeared to have died from loss of blood due to the uncontrolled mite population.

The initial effects of Dri-Die 67 were somewhat more pronounced than those of 18706, but this substance, too, failed to effect eradication and mites were still found crawling on the snakes in both Dri-Die treated cages after 192 hours (Text-figs. 2 & 3). As with 18706, the relative lack of success with Dri-Die 67 in cage II may be due in large part to the high initial infestation. However, comparison with the controls indicates no significant effect in cage II subsequent to the 24-hour observation. After the initial population drop in cage VII, the mite population remained at a fairly low and steady level, but did not disappear. This continuing low infestation may have been due in part to continual re-infestation from the control cage directly above it. Because of these factors, the testing of the mite-eradicating abilities was biased against both Dri-Die 67 and 18706 and should not be construed as proof that these products are necessarily inferior. However, in both Dri-Die treated cages the snakes were obviously dehydrated and consumed considerable quantities of water. The death of one juvenile garter snake in cage VII may have been accidental, but the deaths of six out of ten snakes in cage II must be attributed, directly or indirectly, to the Dri-Die treatment. All of the smaller snakes died and it appeared that dehydration and exsanguination were the primary causes of death. There were indications that the mites, desiccated by Dri-Die, were stimulated to feed continuously. The snakes, despite the fact they they were constantly drinking water, were unable to maintain a proper balance of body fluids and succumbed.

Although the four cages (I, III, VI and VIII) that were treated with Diazinon 25-E or Ortho Dibrom 8-E were favored from the standpoint of position and initial infestation (Text-figs. 1 & 2), the results were so dramatic as to be unquestionably significant. The mite populations in all four cages appeared to be completely or almost completely destroyed in 24 hours. Complete eradication apppeared to have been accomplished in cages I and III by 24 hours, although a singe mite was found in cage I at 168 hours and in cage III at 24 hours. These mites and the very few mites that appeared in cages VI and VIII after the initial drop may have been migrants from the other cages and these may not have had time to succumb. It cannot be concluded that either Diazinon 25-E or Ortho Dibrom 8-E will guarantee eradication of the snake mite, but both compounds appear extremely promising. All of the snakes treated with either of these two acaricides survived the experiment and appeared to be in good health. Subsequent use of Diazinon 25-E in the laboratory at The University of Kansas has proved completely effective against the snake mite in cages of snakes that are kept in the same room and on the same shelf with two untreated cages, which contain thriving experimental colonies of Ophionyssus natricis.

#### SUMMARY AND CONCLUSIONS

Forty-five materials were each tested initially against samples of ten engorged adult female snake mites. Eleven of the products killed all ten mites in less than 24 hours. Nine of these were then tested for toxicity to small reptiles along with three other slower-acting acaricides that had been recommended by amateur herpetologists. Several of the remaining materials achieved significant mite mortality in varying times greater than 24 hours and many of these deserve further testing (Table I).

Only three of the acaricides that were tested for herpeticidal activity proved to be safe for use on small reptiles. These products, American Cyanamid 18706, Ortho Dibrom 8-E and Diazinon 25-E, were then each tested at two concentrations under simulated zoo conditions. Because Dri-Die 67 had achieved such promising results

in other studies (Tarshis, op. cit.), it was also included in the "field" test.

The disproportionately large mite populations to which American Cyanamid 18706 was subjected biased the "field" test so that the results of this test were inconclusive with regard to this acaricide. The compound appears to be of potential value in the control of the snake mite and should be further examined. The test was similarly biased against Dri-Die 67, but, while earlier tests showed this aerogel to be highly effective against the snake mite, the results were erratic and there was strong evidence that Dri-Die 67 may sometimes be detrimental to small snakes.

The results of all tests with Ortho Dibrom 8-E and Diazinon 25-E were quite dramatic and very encouraging. Both of these acaricides appear to be capable of eradicating snake mite infestations within 24 to 48 hours without harming the reptile hosts. Both are emulsifiable in water and can be sprayed directly into the cage and on the reptiles, necessitating no special or additional maintenance operations. Ortho Dibrom may be applied in concentrations of 2 to 4 cc. per gallon of water and Diazinon in concentrations of one part of the 25% concentrate in 240 to 480 parts of water. If the first application does not completely eradicate an established infestation, a second application in two to four weeks should accomplish this. After ridding a collection of the original infestation, it should be possible to keep the collection free of mites by instituting a program of rigid quarantine for all incoming reptiles with routine spraying of these with either Dibrom or Diazinon before introducing them into the collection.

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